ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.653

Volume 130, pp. 653–660 July–September 2015

Artomyces nothofagi sp. nov., a clavarioid fungus from a Chilean *Nothofagus* forest

Richard Kneal[1] & Matthew E. Smith*[1]

[1]*Department of Plant Pathology, University of Florida, Gainesville, FL, 32611, USA*

*Correspondence to: *trufflesmith@ufl.edu*

Abstract – We collected an *Artomyces* species on highly decayed wood of *Nothofagus dombeyi* in southern Chile that did not match the description of any previously described species. Here we describe this fungus as *Artomyces nothofagi* based on a combination of unique morphological features and a distinct nrITS DNA sequence.

Key words – *Auriscalpiaceae*, coral fungi, Patagonia, *Russulales*

Introduction

In the past, coral fungi were treated as a single taxonomic unit because all coralloid and clavarioid fungi were thought to be closely related (e.g., Coker 1923, Corner 1950). However, recent molecular phylogenetic results indicate that coralloid and clavarioid fungi are polyphyletic and are spread across many different orders of *Agaricomycotina* (Pine et al. 1999, Hibbett 2007). Jülich (1982) erected the genus *Artomyces* Jülich to accommodate clavarioid species with simple to pyxidately branched basidiomata, hymenial gloeocystidia, gloeoplerous hyphae, and small, hyaline, amyloid basidiospores. In a molecular phylogenetic overview of the *Russulales*, Miller et al. (2006) included *Artomyces* in their circumscription of *Auriscalpiaceae* where it comprises a clade sister to the other genera in that family, including *Auriscalpium* Gray, *Lentinellus* P. Karst., *Gloiodon* P. Karst., and *Dentipratulum* Domański.

Lickey et al. (2003) provided the only comprehensive phylogenetic and taxonomic study of *Artomyces*. This study supported Jülich's (1982) interpretation of *Artomyces* as a distinct genus that is separate from *Clavicorona* Doty. Lickey et al. (2003) formally described seven new species, created a new combination based on *Clavicorona microspora* Qiu X. Wu & R.H. Petersen, and provisionally described two additional unnamed taxa. Overall, Lickey et al. (2003) accepted 15 species within *Artomyces*, based on a combination of morphology, nuclear ribosomal internal transcribed spacer DNA (nrITS DNA) sequences, and mating studies. No new *Artomyces* species have been described since this comprehensive work.

During a fungal foray near the base of the Melimoyu volcano in southern Chile, we encountered a clavarioid basidiomycete in the genus that was fruiting on highly decayed wood of *Nothofagus dombeyi* (Mirb.) Oerst. This clavarioid fungus, which did not match any described *Artomyces* species, had a unique nrITS DNA sequence. We describe this fungus here as *Artomyces nothofagi.*

Materials & methods

Colors of fresh and dried specimens are described here in general terms. Microscopic features were described from dried material mounted in 5% KOH, Melzer's reagent, Congo Red, or water for standard light microscopy. Microscopical observations were made using a Nikon Optiphot microscope. Basidiospores were measured from mature basidiocarps that were shaved into thin slices or crush mounted and then stained in Melzer's reagent. Spore statistics were calculated from measurements from 20 spores as length × width. Length : width ratios are designated as Q; L_m = mean length; W_m = mean width; and Q_m = mean Q. At least 10 structures were measured for each of the other anatomical characteristics. Basidia, generative hyphae, gloeoplerous hyphae and gloeocystidia were observed and measured in crush mounts with 5% KOH, Congo Red and water. Specimens are conserved in Herbario, Museo Nacional de Historia Natural, Santiago, Chile (SGO) and University of Florida Herbarium, Gainesville, U.S.A. (FLAS).

Fresh basidiocarp tissue was preserved in the field with CTAB buffer. DNA was extracted by a modified CTAB method followed by PCR of the nrITS DNA region using the primers ITS1F and ITS4 according to published protocols (Gardes & Bruns 1993). PCR products were visualized on 1.5% agarose gels stained with SYBR Green I (Molecular Probes, Eugene, OR, USA). Successful amplicons were cleaned with ExoSAP-IT (U.S.B. Corporation, Cleveland, OH, USA). Bidirectional sequencing was performed with ITS1F and ITS4 by the University of Florida ICBR sequencing center (www.biotech.ufl.edu). Sequences were edited with Sequencher v.4.1 (Gene Codes Inc., Ann Arbor, MI, USA).

All available *Artomyces* nrITS DNA sequences were downloaded from GenBank and combined with our sequence into an alignment using Mesquite v.1.1 (Maddison & Maddison 2006). Preliminary sequence alignments were performed with MUSCLE (Edgar 2004) followed by manual adjustments in Mesquite. A maximum parsimony (MP) heuristic search was conducted with PAUP* v.4.0a109 (Swofford 2002) using 100 random addition replicates and TBR branch swapping. MP bootstrapping was used to assess support for topology using 1000 replicates with 10 random addition replicates each. Maximum likelihood analysis was performed using GARLI (Zwickl 2006) with the GTR+I+G model followed by ML bootstrapping with 1000 bootstrap replicates.

Results

Morphological analysis of *Artomyces nothofagi* indicates that this species is distinct from all other described species based on a combination of basidiomata

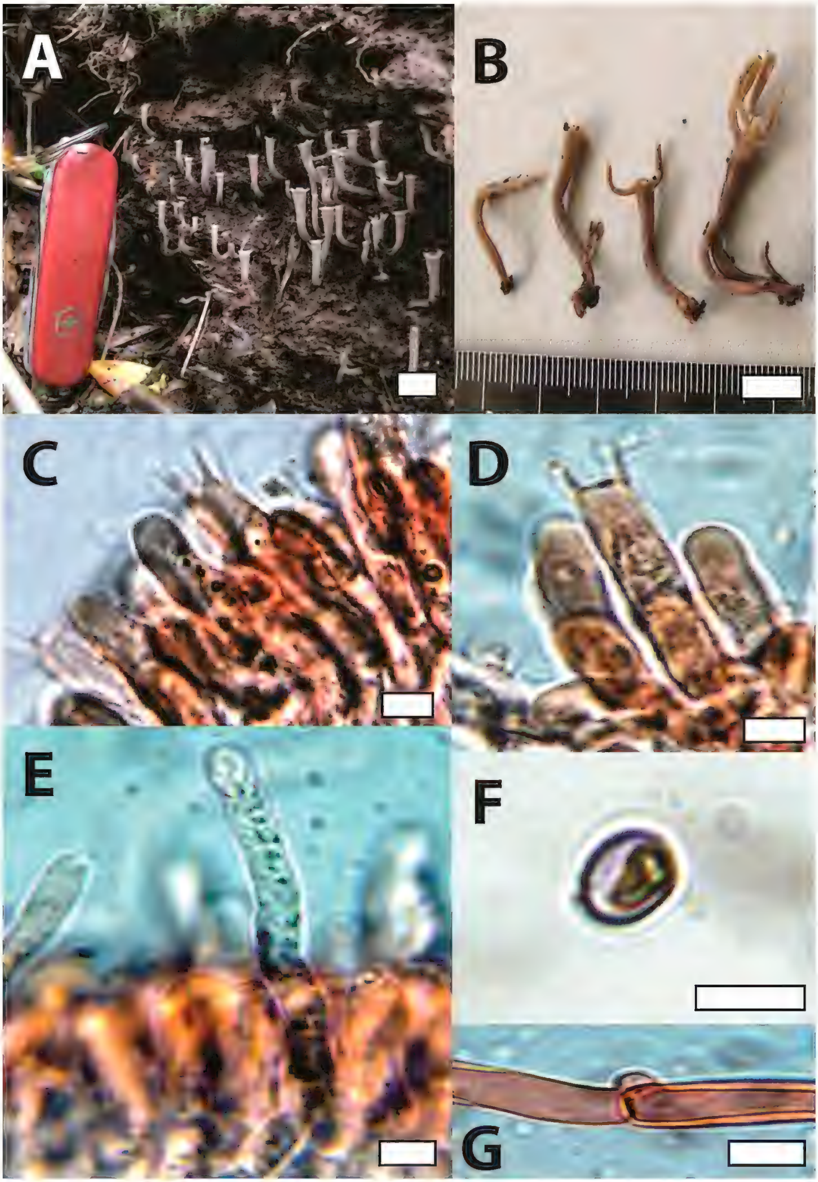

Figure 1. *Artomyces nothofagi* (holotype, SGO 164746; isotype, FLAS-F-58994): A. Basidiomata fruiting directly on decayed wood of *Nothofagus dombeyi* in southern Chile. B. Fresh basidiomata on grey background. C. 4-spored basidia. D. 2-spored basidia. E. Gloeocystidia. F. Guttulate, amyloid basidiospores. G. Clamp connection on generative hyphae. Images C–E and G mounted in KOH and Congo Red; image F mounted in Melzer's reagent. Scale bars: A = 2.5 cm; B = 1 cm; C–G = 5 µm.

shape, size, and color, spore size and ornamentation, and morphology of the gloeocystidia (Fig. 1). Additionally, molecular phylogenetic analysis shows *A. nothofagi* with a unique nrITS DNA sequence, supporting it as phylogenetically distinct from all other *Artomyces* species. Although *A. nothofagi* clearly belongs to *Artomyces,* our ML and MP analyses did not provide bootstrap support for a close relationship with any of the other species. Nonetheless, the best ML tree (Fig. 2) and best MP trees (data not shown) consistently resolved *Artomyces nothofagi* in an unsupported clade with *A. colensoi* (Berk.) Jülich and *A. stephenii* Lickey.

Taxonomy

Artomyces nothofagi M.E. Sm. & Kneal, **sp. nov.** Fig. 1

MycoBank 810369

> Differs from other *Artomyces* species by its combination of smooth basidiospores, mostly unbranched basidiomata, gloeocystidia that protrude well past the hymenium, and a unique nrITS DNA sequence.
>
> Type: Chile, XI Región Aysén del General Carlos Ibáñez del Campo, Melimoyu reserve, near Pacific Ocean at the base of Volcan Melimoyu, Tres Lagunas trail 44°05′14.5″S 73°04′58.9″W, 14 March 2012, M.E. Smith MES559 (**Holotype**, SGO164746; **isotype**, FLAS-F-58994; GenBank KP025706).
>
> Etymology: The epithet *nothofagi* refers to the substrate species, *Nothofagus dombeyi.*

Basidiomata lignicolous, scattered to gregarious, ≤50 mm tall × 15 mm wide at the apex; generally unbranched and coronate, rarely forming cristate branches at the apices; apices coronate-cristate to cuspidate and dull grey to faintly brownish with occasional vinaceous tones; when dried black to caramel brown; usually lacking obvious basal mycelium, but occasionally with whitish basal mycelium. Odor sweet and pleasant, reminiscent of apricots; taste not recorded.

Context composed of: (1) generative hyphae, thin walled, usually encrusted with yellow-brown material, 2.0–15.0 µm diam.; clamp connections present; and (2) gloeoplerous hyphae, common in the trama, aseptate, 2.5–8.0 µm diam., terminating in the hymenium as gloeocystidia. Gloeocystidia common, mostly cylindrical to clavate but occasionally fusiform, flexuous or subcapitate to capitate, 2.5–6.5 µm diam., consistently narrowing within the subhymenium, non-emergent to protruding 8.0–25.0(–50) µm. Basidia (2–)4-sterigmate, narrowly clavate to cylindrical, with obvious basal clamp connections, 20.8–25.6 × 4.7–6.5 µm ($L_m \times W_m = 22.5 \times 5.4$ µm) not including sterigmata; sterigmata 2.6–4.3 µm long, ($L_m = 3.2$ µm). Basidiospores 4.0–5.2 × 3.4–4.5 µm ($L_m \times W_m = 4.7 \times 3.7$ µm), Q = 1.0–1.4 ($Q_m = 1.3$), globose to subglobose, smooth, hyaline, distinctly amyloid in Melzer's reagent; contents with a single large guttule; hilar appendage present.

Ecology & distribution – Known only from the type collection. Clustered to gregarious on highly decayed wood of *Nothofagus dombeyi* in southern Chile; March.

Discussion

Both morphology and molecular phylogenetic analysis support *Artomyces nothofagi* as a distinct species. The petite, mostly unbranched basidiomata of this species along with the smooth globose to subglobose basidiospores are unusual features within *Artomyces*. The frequently encrusted generative hyphae, basidia that are sometimes two-spored, and gloeocystidia that protrude well past the hymenium (≤50 µm) are also distinctive within the genus (Fig. 1). *Artomyces nothofagi* has smooth spores, which contrasts with 14 (out of 15) of the other species characterized by spores that are clearly ornamented when viewed with light microscopy. This new species is also unusual because its basidiomata are mostly unbranched and coronate, only rarely forming cristate branches at the apices. In contrast, most described *Artomyces* species have basidiomata that branch regularly more than twice.

The most morphologically similar species to *A. nothofagi* is *A. cristatus* (Kauffman) Jülich, which is known from conifer wood in Canada, northern Europe, and the USA. Both species have unbranched basidiomata and smooth basidiospores with large guttules. However, the spores of *A. cristatus* are distinctly cylindrical, not globose to subglobose as in *A. nothofagi.* In addition, the gloeocystidia of *A. cristatus* protrude only ≤6 µm past the hymenium whereas those of *A. nothofagi* protrude ≤50 µm. Based on the habitat and several distinct morphological differences, it is evident that these are indeed two different species.

The only two *Artomyces* species previously known from southern South America, *A. adrienneae* Lickey and *A. austropiperatus* Lickey, are also morphologically distinct. The basidiomata of *A. adrienneae* are "pure white or white mellowing to a fleshy buff" and regularly branched whereas those of *A. nothofagi* are much darker and rarely branched. Moreover, *A. adrienneae* basidiospores are ornamented (asperulate) and narrower than *A. nothofagi* spores. *Artomyces adrienneae* also lacks the encrusted generative hyphae (Lickey et al. 2003) that are common in *A. nothofagi.*

Artomyces austropiperatus is the only other species described from South America, although this species has also been documented from Tasmania and the North Island of New Zealand. Although *A. austropiperatus* is similar to *A. nothofagi* in the sizes of the gloeocystidia, basidia, and spores, *A. austropiperatus* spores are ornamented ("obviously asperulate") whereas those of *A. nothofagi* are smooth. Also, Lickey et al. (2003) describes

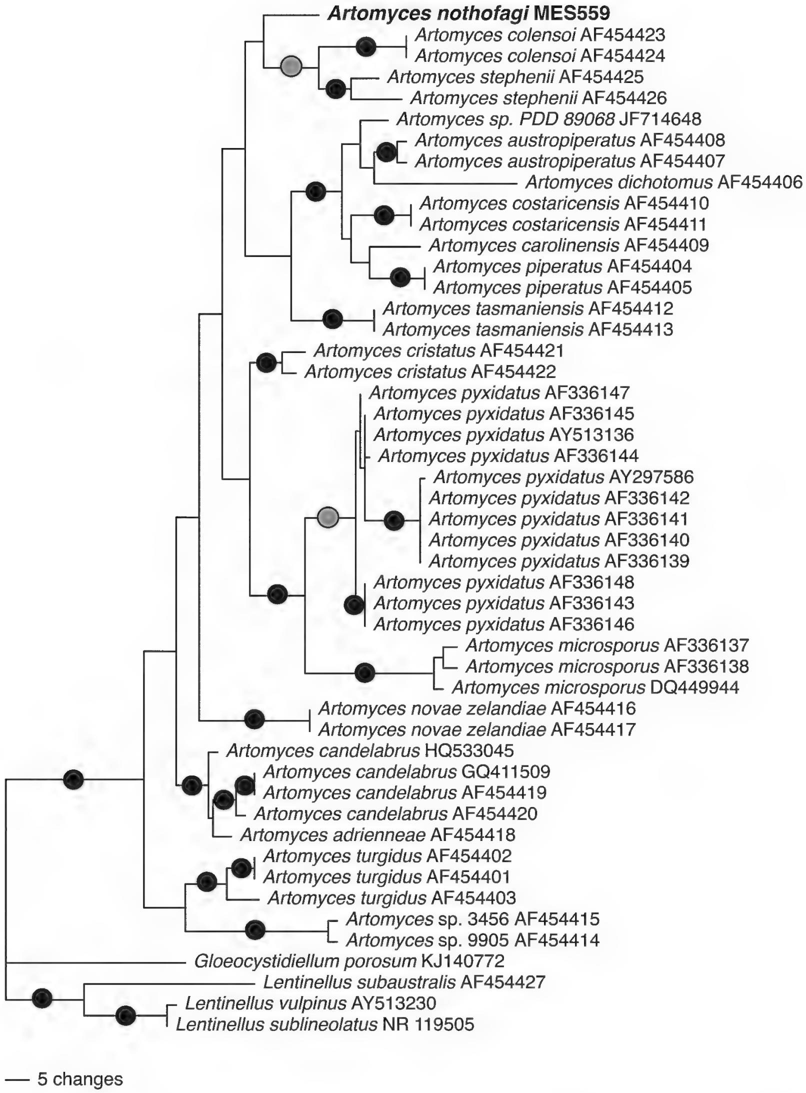
Artomyces nothofagi MES559
Artomyces colensoi AF454423
Artomyces colensoi AF454424
Artomyces stephenii AF454425
Artomyces stephenii AF454426
Artomyces sp. PDD 89068 JF714648
Artomyces austropiperatus AF454408
Artomyces austropiperatus AF454407
Artomyces dichotomus AF454406
Artomyces costaricensis AF454410
Artomyces costaricensis AF454411
Artomyces carolinensis AF454409
Artomyces piperatus AF454404
Artomyces piperatus AF454405
Artomyces tasmaniensis AF454412
Artomyces tasmaniensis AF454413
Artomyces cristatus AF454421
Artomyces cristatus AF454422
Artomyces pyxidatus AF336147
Artomyces pyxidatus AF336145
Artomyces pyxidatus AY513136
Artomyces pyxidatus AF336144
Artomyces pyxidatus AY297586
Artomyces pyxidatus AF336142
Artomyces pyxidatus AF336141
Artomyces pyxidatus AF336140
Artomyces pyxidatus AF336139
Artomyces pyxidatus AF336148
Artomyces pyxidatus AF336143
Artomyces pyxidatus AF336146
Artomyces microsporus AF336137
Artomyces microsporus AF336138
Artomyces microsporus DQ449944
Artomyces novae zelandiae AF454416
Artomyces novae zelandiae AF454417
Artomyces candelabrus HQ533045
Artomyces candelabrus GQ411509
Artomyces candelabrus AF454419
Artomyces candelabrus AF454420
Artomyces adrienneae AF454418
Artomyces turgidus AF454402
Artomyces turgidus AF454401
Artomyces turgidus AF454403
Artomyces sp. 3456 AF454415
Artomyces sp. 9905 AF454414
Gloeocystidiellum porosum KJ140772
Lentinellus subaustralis AF454427
Lentinellus vulpinus AY513230
Lentinellus sublineolatus NR 119505
5 changes

FIGURE 2. Maximum likelihood phylogeny (–ln 3515.31912) of *Artomyces* based on internal transcribed spacer ribosomal DNA. Nodes with both maximum likelihood and maximum parsimony bootstrap support values ≥75 are depicted with solid black circles whereas nodes that are supported by only one of these two methods are depicted with grey circles. *Gloeocystidiellum porosum* and three species of *Lentinellus* were used as outgroups.

A. austropiperatus as "well-branched, more or less compact and stoutish," and *A. austropiperatus* also lacks encrusted generative hyphae.

Although *A. nothofagi* consistently clustered with *A. stephenii* and *A. colensoi* in both our ML and MP analyses, there was no bootstrap support for this relationship. Furthermore, *A. nothofagi* is morphologically distinct from both species. *Artomyces stephenii* forms relatively large basidiomata and is known only from hardwood substrates in Costa Rica. It is described as "reminiscent of *A. pyxidatus*" and is "often profusely branched and compact" (Lickey et al. 2003). Microscopically, *A. stephenii* is described with "minutely asperulate, weakly amyloid" basidiospores whereas the spores of *A. nothofagi* are smooth, distinctly amyloid, and larger than those of *A. stephenii* (Lickey et al. 2003). Also, the gloeocystidia of *A. stephenii* are described as protruding only ≤12 µm past the hymenium, not ≤50 µm as occurs frequently in *A. nothofagi.*

Artomyces nothofagi is also morphologically distinct from *A. colensoi. Artomyces colensoi* is an Australasian species known from hardwood logs in New Zealand and southern Australia. This species was described as "well-branched" and having a slightly peppery odor (Lickey et al. 2003) that contrasts with the sweet, pleasant odor of *A. nothofagi. Artomyces colensoi* also has smaller, more ellipsoid spores, with $Q_m = 1.48$ (cf. $Q_m = 1.3$ for *A. nothofagi*). Finally, *A. colensoi* is described as having "some encrusted" generative hyphae, "a character not observed in the type specimen" (Lickey et al. 2003), whereas in *A. nothofagi* the generative hyphae are frequently encrusted.

Acknowledgements

A special thanks is due to the conservation and sustainable development organization Patagonia Sur (www.patagoniasur.com) for facilitating access to the Melimoyu Reserve where *A. nothofagi* was collected. We also thank Dr. Donald H. Pfister and Harvard University's David Rockefeller Center for Latin American studies for travel funding and logistical planning that made this work possible. Funding for this work was provided in part by National Science Foundation grant DEB-1354802 (to MES) and also by the University of Florida's Institute for Food and Agricultural Sciences (IFAS). We would also like to thank Dr. Ronald Petersen and Dr. Edgar Lickey for providing valuable reviews and feedback that improved this paper.

Literature cited

Coker WC. 1923. The clavarias of the United States and Canada. The University of North Carolina Press, North Carolina.

Corner EJH. 1950. A monograph of *Clavaria* and allied genera. Annals of Botany Memoirs 1. 740 p.

Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5: 113. http://dx.doi.org/10.1186/1471-2105-5-113

Gardes M, Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes-applications to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x

Hibbett DS. 2007. After the gold rush, or before the flood? Evolutionary morphology of mushroom-forming fungi (*Agaricomycetes*) in the early 21st century. Mycological Research 111: 1001–1018. http://dx.doi.org/10.1016/j.mycres.2007.01.012

Jülich W. 1982 ["1981"]. Higher taxa of *Basidiomycetes.* Bibliotheca Mycologica 85. 485 p.

Lickey EB, Hughes KW, Petersen RH. 2003. Phylogenetic and taxonomic studies in *Artomyces* and *Clavicorona* (*Homobasidiomycetes: Auriscalpium*). Sydowia. 55(2): 181–254.

Maddison WP, Maddison DR. 2006. Mesquite: a modular system for evolutionary analysis. Version 1.11. http://mesquiteproject.org

Miller SL, Larsson E, Larsson KH, Verbeken A, Nuytinck J. 2006. Perspectives in the new *Russulales.* Mycologia 98(6): 960–970. http://dx.doi.org/10.3852/mycologia.98.6.960

Pine, EM, Hibbett DS, Donoghue, MJ. 1999. Phylogenetic relationships of cantharelloid and clavarioid homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. Mycologia 91(6): 944–963. http://dx.doi.org/10.2307/3761626

Swofford DL. 2009. Phylogenetic Analysis Using Parsimony (*and other methods) ver 4.0a109. Sinauer Associates, Sunderland, Massachusetts.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. University of Texas Press, Austin, Texas.